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Correspondence

Questioning *in Situ* PCR

To the Editor-in-Chief:

I am writing in reference to the paper by R. H. Chen and S. V. Fuggle, "*In Situ* cDNA Polymerase Chain Reaction: A Novel Technique for Detecting mRNA Expression" (Am J Pathol 1993, 143:1527). First, the term "novel" implies an original idea. At least six peer-reviewed papers have appeared prior to the acceptance date of their article which describes the *in situ* detection of PCR-amplified cDNA (four published by my group). I would be glad to supply this list to them. Furthermore, the RT *in situ* PCR protocol was listed in 1992 in the book *PCR* in Situ *Hybridization: Protocols and Applications* (New York, Raven Press), written by me and published in 1992. Although the authors are to be congratulated for successfully performing RT *in situ* PCR, I strongly believe it is important to acknowledge those before you who were able to accomplish similar work, especially in a methods paper such as this.

Second, I would like to make the authors aware of the following paper: Nuovo GJ, et al: Importance of different variables for optimizing *in situ* detection of PCR-amplified DNA. PCR Methods Appl 1993, 2:305–312. This paper shows how the PCR product can migrate from the cell with overprotease digestion. It also shows how important it is to use a higher concentration of $MgCl_2$ and the need for BSA when compared with solution phase PCR. The authors arrive at the same conclusions toward the end of their discussion, although I see no data in their work on varying the $MgCl_2$ concentration, and they do not include BSA in their PCR reagent list in the Materials and Methods section.

I trust the authors will be glad to acknowledge the work of others published prior to their acceptance date concerning RT *in situ* PCR, including two papers published in 1992. I also think they should include such basic data as the optimization of $MgCl_2$ and the use of BSA in the Results section.

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Authors' Reply:

We are writing in reply to Dr. Nuovo's comments about our paper. In that paper we reported our development of a technique for PCR amplification and detection of intracellular message. This manuscript was submitted in the "Short Communications" category and as such there are constraints on the amount of space permitted. In our initial protocol we did not use BSA as a component of the PCR reaction mixture. We demonstrated that the conditions for protease digestion are quite important, as extended digestion decreases signal intensity. Further, we noticed that the *in situ* cDNA PCR reaction often requires a higher concentration of $MgCl_2$ than that required for tube PCR. Since the changes in concentration vary according to the primers used, in this brief manuscript we did not list the full spectrum of $MgCl_2$ concentrations tested for each primer pair. The fact that Dr. Nuovo and colleagues have had similar experience with protease digestion and $MgCl_2$ corroborates the point that these variables should be carefully optimized to achieve the best results.

We recognize that other groups have developed their particular protocols for amplifying intracellular signals according to their research interests, and we are happy to acknowledge the contributions that Dr. Nuovo and his colleagues have made to this field. We regret the omission of a reference to their article (PCR Methods Appl 1992, 2:117) and book (*PCR* in Situ *Hybridization: Protocols and Applications* 1992, Raven Press, New York). Embleton and colleagues have also reported in cell PCR from mRNA in which the products were labeled with a fluorochrome and sorted by flow cytometry (Nucleic Acids Res 1992, 21:3821). After the submission of our paper to the *American Journal of Pathology,* a number of other groups have reported their methods of amplifying intracellular message.

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